JAPANESE [JP,09-176010,A]

CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS OPERATION EXAMPLE

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the synthetic inhibitor of the protein in which a molecular weight belongs to the heat shock protein group (it is hereafter called HSP60 family) of a before [from 57 kilodalton (kD) / 68kD] which contains flavonoid as an active principle. Synthetic inhibitor of protein belonging to HSP60 family by this invention, By controlling in-house composition of the protein which belongs to HSP60 family especially, The physiological status of sick patients, such as the autoimmune disease considered that protein belonging to HSP60 family participates in the onset, for example, type I diabetes mellitus, and rheumatoid arthritis, can be made to

be able to improve effectively, and autoimmune diseases, such as type I diabetes mellitus and rheumatoid arthritis, can be treated effectively.

[0002]

[Description of the Prior Art]In recent years, an autoimmune disease poses a big problem. An autoimmune disease is illness which the immune system which should not be attacked to the ingredient which constitutes the self body properly speaking reacts to a self organization, and destroys, for example, type I diabetes mellitus, rheumatoid arthritis, etc. are included. for example, -- in our country, a diabetic increases remarkably in recent years with development of economy, society, and culture, improvement in a living standard, or change of a lifestyle -- symptoms -- serious-illness-izing -- it has complicated. Although a patient's prognosis has improved, in addition, arteriosclerosis was also promoted by progress of diabetes-mellitus study, and a characteristic retinopathy, a nephropathy, and neuropathy have interfered [great] with health and a social activity by it.The incidence rate of type I diabetes mellitus (insulin dependent diabetes mellitus; insulin-dependent diabetes mellitus;IDDM) among diabetes mellitus, It increases several times in these years of tens of in many countries, and it is expected that it will suffer from it to type I diabetes mellitus by the time 1% of Homo sapiens living now will be 70 years old.

[0003]Type I diabetes mellitus is a disease which will be in an insulin deficiency state since only the beta cell of pancreas Langerhans' islet which is an insulin production cell is destroyed in autoimmunity, an organ -- it is a specific autoimmune disease (Atkinson -- M . -- A . -- et al . -- : -- -- "Sci. Am."263: 42-49 and 1990; Todd JA.:[ ]" Immunol. Today", 11: 122-129, and 1990). The autoimmune process in which type I diabetes mellitus is caused is restricted only to the pancreas very strictly.

Before often growing up, symptoms develop in many cases. When the symptoms of type I diabetes mellitus develop clinically, there is inflammation (insulitis) of a pancreatic islet and most beta cells which are producing the insulin are lost specifically (263: ["Sci. Am." and] et al.[Atkinson, M. A., and]: 62-67, 1990). Diabetic clinical symptoms will appear for the first time, after being destroyed even to such an extent that most beta cells (probably not less than 90%)

could not be reproduced, and a patient's survival will be dependent on external supply of an insulin. That is, this autoimmune reaction has already done irreversible damage at the stage which can be discovered by a clinical diagnosis. And type I diabetes mellitus includes many problems -- the many do not show a remarkable subjective sign.

[0004]Rheumatoid arthritis is a chronic-inflammation nature disease which makes a joint synovial membrane the main seat of a lesion. As for a lesion region, it is not rare not to sometimes remain only in a joint synovial membrane, but to attain to the whole body, either. The inflammation which carried out initiation to the joint synovial membrane causes destruction of synovial membrane growth and also a cartilage, and a bone soon, and destruction of a joint organization is caused. As a result, he a patient not only receives restriction socially, domestically, and remarkably, but cannot ignore an economic burden. The patient number of rheumatoid arthritis is made into 0.1 to 0.3% of population. This is an example of a definite diagnosis of rheumatoid arthritis, and it seems that a patient number will increase also before and after the 10 times if the circumference disease of the example of a suspicious diagnosis, etc. and rheumatoid arthritis is included.
[0005]On the other hand, a heat shock protein (it is also called heat shock protein;HSP and stress protein), a group revealed by the cell by stimulating a cell by a certain stress, for example, heat, a heavy metal, drugs, an amino acid analog, or hypoxia (low concentration oxygen) -- it is protein. The heat shock protein exists in the nature universally.
It is produced by bacteria, yeast, vegetation, an insect, and the higher animal including Homo sapiens.

[0006]Although the kind is various, HSP, From the size of a molecular weight to HSP90 family (for example, HSP of 90kD or 110kD, etc.). It can divide roughly into 4 of HSP70 family, HSP(s)(for example, HSP of 70 - 73kD, etc.)60 family, and low molecule HSP(s) (for example, HSP of 57 - 68kD, etc.) families (for example, HSP of 20kD, 25 - 28kD, or 47kD, etc.) families. In this specification, the number indicated to be HSP immediately after that shall show HSP which has specific molecular weight, for example, HSP of molecular weight 60kD shall be called "HSP60." As

mentioned above, although many kinds exist in HSP, these differ not only in a molecular weight but in structure, a function, or character etc., respectively. the response to stress -- in addition, some of these protein being compounded compositionally and under normal environment, A thing like a meeting of proteinic folding, unfolding, and a protein subunit and proteinic membrane transport for which the indispensable physiological role is played is shown. These functions as a heat shock protein are called a molecular chaperone.

[0007]One of the being observed about the cause of a disease of an autoimmune disease has molecule homology (molecular mimicry). Namely, when the self-antigen has an extraneous antigen and common antigenicity, such as a microorganism, The result in which the antibody generated by microbial infection and a sensitized lymphocyte attack a self organization by a cross reaction, It is thought that the symptoms of an autoimmune disease develop (248: 263: ["Sci. Am." and] 42-49, 1990;Shinha, A. A. et al.: "Science"). [M. A. et al/ Atkinson and /.:] 1380-1388, 1990. For example, protein belonging to HSP60 bacterial family, They are main antigens, such as tuberculosis, a leprosy, syphilis, Legionnaires' disease, or Lyme disease (Young, R. A. et al.: "Cell", 59:5-8, 1989), And it is thought that the autoimmune disease by the molecule homology from whom an infectious disease became a trigger shows the symptoms of protein belonging to HSP60 bacterial family since it has strong immunogenicity and has molecule homology with self protein (Homo sapiens who is a host).

[0008]for example, the antibody (64kD autoantibody) reacted to 64kD protein detected in a diabetic patient or the family's blood -- a beta cell -- it is easy to appear well [just before / specific / carrying out and being diagnosed as diabetes mellitus]. That is, the islet cell antigen considered to be the cause of the onset in type I diabetes mellitus is glycoprotein (Baekkeskov, S. et al.: "Nature", 298: 167-169, 1982) of molecular weight 64kD. The antibodies to 64kD protein are not only human diabetes mellitus but (Atkinson, and M. A. et al. : "Lancet", 335 : 1357-1360, 1990), BB rat (S. Baekkeskov) et al.: "Science", 224: Like 1348-1350, and 1984 and a NOD mouse (Atkinson, M. A. et al.: "Diabetes", 37: 1587-1590, 1988), It is detected also in the model animal of type I diabetes mellitus which shows the

symptoms of type I diabetes mellitus automatically and in which many features of human type I diabetes mellitus are shown. Since 64kD protein of the pancreas beta cell in type I diabetes mellitus is derived by cytokine or thermal stimulation, it may be a heat shock protein.

[0009]64kD protein of the pancreas Langerhans' islet beta cell in the NOD mouse which is a model animal of type I diabetes mellitus, It is shown that it is a self-antigen to which cross reaction nature is immunologically indicated to be an antibody to protein belonging to HSP60 family of a tubercule bacillus (Mycobacterium tuberculosis). Thus, by observing immunological decussation between protein and 64kD protein self-antigen belonging to HSP60 family, 64kD protein of a pancreas beta cell may be a member of HSP60 family, and it is suggested that the mechanism of the autoimmunity which intersects the epitope of protein belonging to HSP60 family participates in the onset of type I diabetes mellitus. An import of the clone of the T lymphocyte which has singularity in protein belonging to HSP60 family of a tubercule bacillus will cause the Langerhans' islet flame and hyperglycemia in a young NOD mouse. If protein belonging to HSP60 family of a tubercule bacillus is injected into a NOD mouse with a medication method with immunogenicity, i.e., an adjuvant, The symptoms of diabetes mellitus may be made to show at an early stage (87: [Elias, D. et al.:"Proc. Natl. Acad. Sci. USA" and] 1576-1580, 1990). These facts that the immunoreaction of an animal to protein belonging to HSP60 family of Mycobacteria causes type I diabetes mellitus, The attack by the immune system over the antigen which carries out a cross reaction to the antibody to protein belonging to HSP60 family of Mycobacteria shows that an obstacle is done to a beta cell.

[0010]It is known that protein belonging to HSP60 family relates to the adjuvant arthritis of the rat which is the animal model of rheumatoid arthritis, and a human rheumatic arthritis. For example, in the case of rheumatoid arthritis, it is clear protein's which belongs to HSP60 family also in the heat shock protein which is bacterial biomass protein to have the proteoglycan which exists in an articular cartilage, and molecule homology. The intervention of the protein reactivity T lymphocyte belonging to HSP60 family is shown by the adjuvant arthritis of the rat (145: ["Curr. Top.

Microbiol. Immunol." and] 27-83, 1989). This disease by introducing the clone of a reactant T lymphocyte to the protein which received radiation irradiation and which belongs to HSP60 family of a tubercule bacillus immunologically at a defenseless (native) rat, It was found out that it is movable to said rat ("Science", 219: 56-58, 1983; "Nature", 331: 171-173, 1988). This T lymphocyte indicates cross reaction nature to be also the proteoglycan of a joint simultaneously ("Proc. Natl. Acad. Sci. USA", 82: 5117-5120, 1985). The accommodative T lymphocyte derived in protein belonging to this HSP60 family is accepted also by the arthritis by hemolytic streptococcus or Pristane. Therefore, adjuvant arthritis seems to be the autoimmune disease caused by the protein T lymphocyte belonging to anti-HSP60 family. The human juvenile rheumatoid arthritis is also considered in the intervention of the protein reactivity T lymphocyte belonging to HSP60 family.

[0011]The protein which belongs to HSP60 family of Mycobacteria origin out of the synovia of the patient of rheumatoid arthritis is received, The T lymphocyte which reacts specifically is taken out (II["Lancet" and]: 478-480, 1988; "Nature", 339: 226, 1989;"Annu. Rev. Immunol.", 11: 637, 1993). Thus, it receives that protein belonging to HSP60 family of Mycobacteria and the protein in which cross reaction nature is shown are observed in high concentration by the cartilage / pannus joined part of rheumatoid arthritis, It does not accept in a normal organization or the organization which presents the chronic inflammation by other diseases (31: ["Scand. J. Immunol." and] 283-288, 1990). The antibody to protein belonging to HSP60 family also from what is also detected by the rheumatoid arthritis of Homo sapiens and a rat (11: ["Immunol. Today" and] et al.[Kaufmann, S. H. E., and]: 129-136, 1990). It may be said that the cause of a disease of rheumatoid arthritis is the autoimmunity to protein belonging to HSP60 family of Mycobacteria, and the self-antigen with which structure was similar. Therefore, existence of the immune response to protein belonging to HSP60 family relates to the arthritis of both a rat and Homo sapiens.

[0012]

[Problem(s) to be Solved by the Invention]In order to

develop the method of this invention persons improving effectively the physiological status of the patient of autoimmune diseases, such as type I diabetes mellitus and rheumatoid arthritis, in view of the above-mentioned situation, and treating those autoimmune diseases effectively, Examination was variously come in piles about the compound in which synthetic depressant action is shown to protein belonging to HSP60 family. As a result, this invention persons found out controlling specifically composition of protein belonging to HSP60 family in the cell of the organization which flavonoid shows symptoms also unexpectedly. That is, by prescribing flavonoid for the patient, composition of protein belonging to HSP60 intracellular family was controlled, therefore it found out that the therapy of autoimmune diseases, such as type I diabetes mellitus and rheumatoid arthritis, was possible. This invention is based on such knowledge.
The purpose is to provide the synthetic inhibitor of protein belonging to HSP60 family which can treat effectively autoimmune diseases, such as type I diabetes mellitus and rheumatoid arthritis.

[0013]
[Means for Solving the Problem]Therefore, this invention relates to synthetic inhibitor of a heat shock protein (namely, protein belonging to HSP60 family) of a before [from a molecular weight of 57 kilodalton containing flavonoid as an active principle / 68 kilodalton]. As for "HSP60 family", a molecular weight as used herein means a heat shock protein group of 57kD - 68kD as aforementioned. As protein belonging to HSP60 family, For example, HSP60 (namely, heat shock protein of molecular weight 60kD), HSP58 (namely, heat shock protein of molecular weight 58kD), HSP65 (namely, heat shock protein of molecular weight 65kD), or GroEL (namely, a molecular weight of a procaryote, for example, Escherichia coli etc., heat shock protein of about 64 kD(s)) can be mentioned.
[0014]
[Embodiment of the Invention]Hereafter, this invention is explained in detail. As an active principle of synthetic inhibitor of this invention, the flavonoid in particular to contain is not limited but publicly known flavonoid can be used for it. As flavonoid used in synthetic inhibitor of this invention, CULCON, flavanones, flavones, flavonols,

FURABANO Norians, flavanols (catechin), isoflavone, or anthocyans can be mentioned, for example. Flavonoid can also be simultaneously used combining several flavonoid which can also use independently or is different. As CULCON, isoOKANIN (Isookanin), the isocarthamin (Isocarthamin), An ISOSA ripple pin (Isosalipurpin), isoBouto Lynn (Isobutrin), Isoliquiritin (Isoliquiritin), OKANIN (Okanin), CULCON (Chalcone), the carthamin (Carthamin), KOREOPUSHIN (Coreopsin), SUCHIROPUSHIJIN (Stillopsidin), Neo SAKURANIN (Neosakuranin), BUTEIN (Butein), PEJISHIN (Pedicin), PEJISERIN (Pedicellin), mallein (Marein), Rance Orrin (Lanceolin), or Rance Ole Ching (Lanceoletin) is illustrated.

[0015]As flavanones, ARUPINECHIN (Alpinetin), isocarthamidin (Isocarthamidin), IsoSAKURANIN (Isosakuranin), the isosakuranetin (Isosakuranetin), IsoPEJISHIN (Isopedicin), eriodictyol (Eriodictyol), Carthamidin (Carthamidin), Cryptostrobin (Cryptostrobin), SAKURANIN (Sakuranin), the sakuranetin (Sakuranetin), SARIPURUPIN (Salipurpin), dihydrowogonin (Dihydrowogonin), Schild MINECHIN (Cyrtominetin), stroboscope PININ (Strobopinin), Naringin (Naringin), the naringenin (Naringenin), The neo carthamin (Neocarthamin), neo hesperidin (Neohesperidin), PINOSUTOROBIN (Pinostrobin), PINOSEMBURIN (Pinocembrin), FARURE roll (Farrerol), butyne (Butin), Bouto Lynn (Butrin), Hula BANOOKANIN (Flavanookanin), the hula BANOMA lane (Flavanomarein), FURABANORANSEORECHIN (Flavanolanceoletin), Flavanone (Flavanone), PURUNIN (Prunin), hesperidin (Hesperidin), Hesperetin (Hesperetin), beret KUNJIN (Verecundin), Gay eriodictyol (Homoeriodictyol), poncy phosphorus (Poncirin), MATTOISHI Norian (Matteucinol), the RIKIRICHI genin (Liquiritigenin), or the liquiritin (Liquiritin) is illustrated.

[0016]As flavones, AKASHIIN (Acaciin), AKASECHIN (Acacetin), Apiin (Apiin), apigenin (Apigenin), wogonin (Wogonin), The OROKI silyne A (Oroxylin-A), the galuteolin (Galuteolin), Chrysin (Chrysin), all [KURISOERI / (Chrysoeriol)], GURUKO luteolin (Glucoluteolin), gene KANIN (Genkwanin), Kos Moshi Inn (Cosmosiin), diosmin (Diosmin), Geos methine (Diosmetin), SUKUTERARIN (Scutellarin), SUKUTERA

lane (Scutellarein), the stroboscope chrysin (Strobochrysin), TEKUTO chrysin (Tectochrysin), the fricin (Tricin), Trun Ginn (Toringin), the nobiletin (Nobiletin), A by Chinese quince (Baicalin), a BAIKA lane (Baicalein), flavone (Flavone), primetin (Primetin), PEKUTORI nari genin (Pectolinarigenin), PEKUTORINARIN (Pectolinarin). PEDARIIN (Pedaliin), PEDARICHIN (Pedalitin), Pons KANECHIN (Ponkanetin), Lina Lynne (Linarin), luteolin (Luteolin), ROIHORIN (Rhoifolin), ROTSUSHIN (Lotusin), or ROTOFURABIN (Lotoflavin) is illustrated.
[0017]As flavonols, aza REACHIN (Azaleatin), the aza lane (Azalein), Astragalin (Astragalin), loon KURARIN (Avicularin), AFUZERIN (Afzelin), AYANIN (Ayanin), IKARIIN (Icariin), Cuttlefish RICHIN (Icaritin), izar PININ (Izalpinin), Isoquercitrin (Isoquercitrin), isorhamnetin (Isorhamnetin), Elian Ching (Erianthin), aura NECHIN (Auranetin), KANUGIN (Kanugin), galangin (Galangin), the faucet gin (Karanjin), Galle Dennin (Gardenin), cannabis citrin (Cannabiscitrin), Xanthorhamnin (Xanthorhamnin), chrysoprase spray NECHIN (Chrysosplenetin), KERUSHITSURON (Quercituron), quercitrin (Quercitrin), quercimeritrin (Quercimeritrin), KERUSETA gitorin (Quercetagitrin), the quercetagetin (Quercetagetin), Quercetin (Quercetin), KEYAKININ (Keyakinin), KENFERIDO (Kaempferid), KENFERITORIN (Kaempferitrin), Kaempferol (Kaempferol), the GOSSHIPI thorin (Gossypitrin), GOSSHIPIN (Gossypin), GOSSHIPECHIN (Gossypetin), SUPIREOSHIDO (Spiraeoside), DACHISU cetin (Datiscetin), TAPUSHIN (Thapsin), the tangeritin (Tangeritin), Tambourine (Tambulin), Tambre Ching (Tambuletin), TERUNACHIN (Ternatin), the trifolin (Trifolin), NARUSHISSHIN (Narcissin), NORUIKARIIN (Noricariin), NORUIKARICHIN (Noricaritin), PATSURECHIN (Patuletin), HIBISU citrin (Hibiscitrin), HIBISU cetin (Hibiscetin), HIPERIN (Hyperin), FISECHIN (Fisetin), flavonol (Flavonol), PERUSHI Chinese quince (Persicarin), the helluva citrin (Herbacitrin), Helluva cetin (Herbacetin), MIKERIANIN (Miquelianin), Myricitrin (Myricitrin), the myricetin (Myricetin), MERACHIN (Meratin), the Melysin pudding (Melisimplin), Melysin plexin (Melisimplexin), MERITERUNACHIN (Meliternatin), MERITERUNIN (Meliternin), morin

(Morin), rum NAJIN (Rhamnazin), Rhamnetin (Rhamnetin), rhamno citrin (Rhamnocitrin), rutin (Rutin), REINOU thorin (Reynoutrin), ROBININ (Robinin), or ROBINECHIN (Robinetin) is illustrated.

[0018]As FURABANO Norians, Astilbin (Astilbin), Al Pinon (Alpinon), AROMADEN drine compounds (Aromadendrin), AMPEROPUCHIN (Ampeloptin), Isoene GERICHIN (Isoengelitin), ene GERICHIN (Engelitin), KEYAKINORU (Keyakinol), dihydroROBINECHIN (Dihydrorobinetin), Stroboscope van KUSHIN (Strobobanksin), the taxifolin (Taxifolin), PINOBANKUSHIN (Pinobanksin), ferra MURIN (Phellamurin), ferra MURECHIN (Phellamuretin), or Fustin (Fustin) is illustrated.

[0019]As flavanols (catechin), AFUZEREKIN (Afzelechin), EPIAFUZEREKIN (Epiafzelechin), the epicatechin (Epicatechin), The epicatechin gallate (Epicatechin gallate), Epigallocatechin (Epigallocatechin), the epigallocatechin gallate (Epigallocatechin gallate), Catechin (Catechin), catechin gallate (Catechin gallate), GAROKATEKIN (Gallocatechin), or the GAROKATEKIN gallate (Gallocatechin gallate) is illustrated.

[0020]As isoflavone, isoflavone (Isoflavon), the irigenin (Irigenin), Ylidyne (Iridin), OSAJIN (Osajin), ONONIN (Ononin), Genistin (Genistin), the genistein (Genistein), Sun Taal (Santal), SOHORABIOSHIDO (Sophorabioside), The sophoricoside (Sophoricoside), daizin (Daidzin), Die zein (Daidzein), the tectorigenin (Tectorigenin), Theque tolidine (Tectoridin), the biochanin A (Biochanin A). PUSOIDOBA petit genin (Pseudobaptigenin), PUSOIDOBAPUCHISHIN (Pseudobaptisin), PURUNU cetin (Prunusetin), prunetin (Prunetin), POMIFERIN (Pomiferin), or the formononetin (Formononetin) is illustrated.

[0021]As anthocyans, AOBANIN (Awobanin), IDEIN (Idaein), Ili Scythia Nin (Ilicicyanin), enin (Oenin), Chrysanthemin (Chrysanthemin), GESUNERIN (Gesnerin), GESUNE lysine (Gesneridin), a keracyanin (Keracyanin), Salvianin (Salvianin), cyanidin (Cyanidin), Cyanine (Cyanin), delphinidin (Delphinidin), Delphinin (Delphinin), the delphin (Delphin), NEGURE theine (Negretein), the violanin (Violanin), Leech SUCHIJIN (Hirsutidin), leech SUCHIN (Hirsutin), Primulin (Primulin), PURUNI cyanine (Prunicyanin), A peonidin (Paeonidin), the peonin

(Paeonin), A petunidine (Petunidin), PETSUNIN (Petunin), a pelargonidin (Pelargonidin), pelargonin (Pelargonin), a malvidin (Malvidin), or the malvin (Malvin) is illustrated.
[0022]As flavonoid used in synthetic inhibitor of this invention, it is quercetin especially preferably. [Namely, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy 4H-1-benzopyran 4-one] Rutin (namely, quercetin 3-rutinoside), a BAIKA lane (namely, 5,6,7-trihydroxy 2-phenyl-4H-1-benzopyran 4-one), or catechin can be mentioned. As catechin used as an active principle in synthetic inhibitor of this invention, (+) Catechin, (+) GAROKATEKIN, (+) catechin gallate, (+) GAROKATEKIN gallate, (-) epicatechin, (-) epigallocatechin, (-) epicatechin gallate, and (-) epigallocatechin gallate are preferred. As flavonoid used as an active principle in synthetic inhibitor of this invention, pure stereoisomeric forms or those mixtures can be used.
[0023]The flavonoid contained in synthetic inhibitor of this invention can be prepared chemosynthesis or by extracting from a natural product and refining. Or a commercial item may be used. The catechin used as an active principle in synthetic inhibitor of this invention is mainly known as tea catechin, when extracting from a natural product and refining, it is not limited to this, but extracting from tea is preferred.
[0024]As mentioned above, since tea catechin is contained in tea, a tea extract can also be used as an active principle of synthetic inhibitor of this invention. In this specification, it is tea as "tea." The entire plant or its part of [Cammellia sinensis, (L) O.Kuntze]. For example, those portions can be used, being able to mean partial fermented material or perfect fermented material, and being independent, or combining [raw /, such as a leaf, xylem, a root, and a fruit, / or a dry matter being able to remain as it is, or] arbitrarily. When using tea leaves as extraction feed, there is a thing of various gestalten, for example, a tea green leaf to finished tea (dry tea), The thing of which stage of the usual tea manufacturing process may be used, and all of non-fermented tea, such as half-fermented tea, such as fermented tea, such as tea, and oolong tea, and green tea, can be used regardless of the grade of fermentation.
[0025]The tea extract which is an active principle of synthetic inhibitor by this invention should just contain the aforementioned tea catechin, therefore a brown crude extract

can be used for it. This tea crude extract can be obtained by warm water's (preferably boiling water's) extracting tea, or extracting using an organic solvent. As an organic solvent, for example Methyl alcohol, ethyl alcohol, Lower alcohol, such as n-propyl alcohol, isopropyl alcohol, or butyl alcohol, Ketone, such as low-grade ester, such as methyl acetate, ethyl acetate, propyl acetate, or butyl acetate, acetone, or methyl isobutyl ketone, can be used, and these organic solvents can be combined independently or suitably, and also it can use by a moisture state anhydrous or preferably.

[0026]It is desirable to carry out heating flowing back at the temperature below the boiling point, being able to use the method used for the usual crude drug extraction as the method of water extraction and organic solvent extraction, for example, stirring to tea-leaves (desiccation) 1 weight section using five to water or organic solvent 20 weight section. The extraction process can shorten extraction time by carrying out preferably for 10 minutes - 24 hours, and usually adding supplementary means, such as stirring, for 5 minutes - seven days, if needed. Water or the organic solvent extract can separate an insoluble matter by suitable methods, such as filtration or centrifugal separation. It is contained in the tea extract for which the output processed further can also use these extracts other than the hot water extract by a conventional method, or an organic solvent extract as an active principle of synthetic inhibitor of this invention with various organic solvents or adsorbent. These tea extracts meet necessity, and it condenses and dries, and disintegration can carry out, and also they can be crystallized and refined from chilled water.

[0027]In this way, the catechin by which the obtained tea extract is contained in tea (especially tea leaves). That is, the impurity which originates in raw material tea simultaneously is included, including tea catechin (for example, catechin, epicatechin, GAROKATEKIN, epigallocatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, or GAROKATEKIN gallate) as a mixture.

[0028]Synthetic inhibitor of this invention is independent [its] about flavonoid or tea extracts, such as the aforementioned quercetin, rutin, a BAIKA lane, or catechin, or -- desirable -- galenical pharmacy -- the usual carrier

permissible-like or in veterinary medicine -- an animal -- mammalian (especially Homo sapiens) can be medicated preferably. Especially as an administration pharmaceutical form, there is no limitation and For example, powder medicine, subtle granules, a granule, Parenterals, such as oral agents, such as a tablet, a capsule, suspension, an emulsion agent, syrups, extracts, or a pill, or injections, liquids for external use, an ointment, suppositories, cream of local administration, or eye drops, can be mentioned. These oral agents, for example Gelatin, sodium alginate, starch, Cornstarch, white soft sugar, milk sugar, grape sugar, mannite, carboxymethyl cellulose, Dextrin, a polyvinyl pyrrolidone, crystalline cellulose, a soybean lecithin, Sucrose, fatty acid ester, talc, magnesium stearate, a polyethylene glycol, In accordance with a conventional method, it can manufacture using excipients, such as a magnesium silicate, a silicic acid anhydride, or synthetic aluminum silicate, a binding material, disintegrator, a surface-active agent, lubricant, a fluid accelerator, a diluent, a preservative, colorant, perfume, corrigent, a stabilizing agent, a moisturizer, an antiseptic, an antioxidant, etc. For example, it is the capsule etc. which were mixed and filled up with catechin 1 weight section and milk sugar 99 weight section.

[0029]As the parenteral administration method, injection (inside of hypodermic and a vein, etc.) or rectum administration is illustrated. In these, injections are used most suitably. For example, in preparation of injections besides flavonoid as an active principle, For example, isotonizing agents, such as nonaqueous solubility solvents, such as water soluble solvents, such as a physiological saline and Ringer's solution, vegetable oil, and fatty acid ester, grape sugar, and sodium chloride, a solubilizing agent, a stabilizing agent, an antiseptic, a suspending agent, an emulsifier, etc. can be used arbitrarily. If an example is shown concretely, after dissolving 10 mg of (+) catechin, and 50 mg of mannitol in distilled water, being referred to as 10 ml and disinfecting with a conventional method, 2 ml is poured distributively to each pint bottle for injection, or it freeze-dries as it is, and is considered as injections. When using it, it dilutes with a physiological saline and is considered as a parenteral solution. Synthetic inhibitor of this invention may be prescribed for the patient using the

technique of a sustained release drug in which the controlled-release polymer etc. were used. For example, synthetic inhibitor of this invention can be made to be able to incorporate into the pellet of ethylene vinyl polymer acetate, and it can transplant surgically during the organization which should treat this pellet.

[0030]Although synthetic inhibitor of this invention is not limited to this, it can contain preferably the salt permitted on flavonoid or its medicine in 0.1 to 80% of the weight of quantity 0.01 to 99% of the weight. Synthetic inhibitor of this invention which contains a tea extract as an active principle can be suitably adjusted so that the flavonoid contained in it may become the aforementioned quantity range, and it can be prepared. It is preferred to pharmaceutical-preparation-ize it using a carrier permissible in galenical pharmacy, in considering the synthetic inhibitor which contains a tea extract as an active principle as the pharmaceutical preparation for internal use. Although the dose in the case of using synthetic inhibitor of this invention changes with a sick kind, a patient's age, the grade of condition, medication methods, etc. and there is no restriction in particular, about 1mg-10g is usually prescribed [as an amount of flavonoid] for the patient in taking orally or parenterally in about 1 to 4 steps per day per adult. It is also possible for a use not to be limited to drugs, either and to give it in the form of ingesta as various uses, for example, functional food, and health food.

[0031]The acute toxicity (LD_{50}) of the quercetin among the flavonoid used for synthetic inhibitor of this invention, In mouse internal use, it is 160 mg/kg (the Merck index, the 11st edition, Merck Co., 1278 pages), and, in the case of a mouse intravenous injection, the acute toxicity (LD_{50}) of rutin is 950 mg/kg (the Merck index, the 11st edition, Merck Co., 1319 pages). Toxicity in particular was not observed in the tea catechin used for synthetic inhibitor of this invention.

[0032]

[Function]As described above, the flavonoid contained in synthetic inhibitor of this invention, Since there is an operation which controls specifically composition of protein belonging to HSP60 intracellular family, if said flavonoid is prescribed for the patient, the biosynthesis of protein belonging to HSP60 family in a cell will decrease specifically. Therefore, protein belonging to HSP60 family

can use said flavonoid for prevention and the therapy of the autoimmune disease relevant to the onset, for example, type I diabetes mellitus, rheumatoid arthritis, etc.

[0033]

[Example]Hereafter, although an example explains this invention concretely, these do not limit the range of this invention.

Example 1: The various Homo sapiens cultured cancer cells below culture of the measurement (1) Homo sapiens cultured cancer cell of the HSP expression amount of the Homo sapiens cultured cancer cell were cultured at 37 ** under 5% carbon dioxide conditions except the time of heat shock processing. Breast cancer cell line MCF7 (ATCC HTB 22) and prostate cancer cell line DU 145 (ATCC HTB 81) were cultivated in the RPMI1640 culture medium which contains inactivation fetal calf serum (it is hereafter called FBS for short) 10%. 10^{-8}Mbeta-estradiol was added to the culture medium of MCF7. Uterus cancer cell line HeLa S3 (ATCC CCL 2.2) and the renal cancer cell strain ACHN (ATCC CRL 1611) were cultivated in the MEM culture medium which includes the inactivation FBS 10%. Prostate cancer cell line PC-3 (ATCC CRL-145) was cultivated in F-12K culture medium (a sigma, catalog number N 3520) which includes the inactivation FBS 7%.

[0034](2) In the culture medium of said various culture human cancer cells two days after flavonoid processing and heat shock processing seeding, any one of the following flavonoid was added and it cultivated for 24 hours. The concentration in the inside of the culture medium after addition of the used flavonoid is quercetin (Nacalai Tesque, catalog number 298-12) 100microM, rutin (Nacalai Tesque, catalog number 303-19) 100microM, and catechin. [The product made by (+)-Catechin; Funakoshi Code No. 0952: EXTRASYNTHESE, France] They were 100microM and BAIKA lane (Matsuura Yakugyo) 20microM. Then, after carrying out heat shock processing for 15 minutes at 45 **, it cultivated at 37 ** all night. The control test was carried out like the above except not adding flavonoid.

[0035](3) Each cell processed for the measurement preceding clause (2) of the HSP expression amount in the Homo sapiens cultured cancer cell was homogenized by the method shown below, and the HSP expression amount was measured in the Western blot technique. That is, it is

phosphate buffered saline about the cell processed for the preceding clause (2). [presentation: The lysis buffer (lysis buffer) after washing by] which below KCl=0.2 g/l, KH_2PO_4=0.2 g/l, NaCl=8 g/l, and Na_2HPO_4(anhydrous) =1.15-g/l; calls PBS (-) [1.0%NP-40, 0.15M sodium chloride, the 50mM tris- HCl (pH 8.0), 5 mM-EDTA, 2 mM-N-ethylmaleimide, 2mM phenylmethyl sulfonylfluoride, 2 microg [/ml] leupeptin, and 2 microg [/ml] pepstatin] 1 ml was added and it settled for 20 minutes in Hikami. Then, centrifugality was performed for 20 minutes at 12000 rpm at 4 **. 10micro of supernatant liquid l after centrifugality was added to 790micro of PBS(-) l, and also 200micro of protein assay stain solutions (Dye Reagent Concentrate : Bio-Rad, catalog number 500-0006) l were added. For 5 minutes, after settling at a room temperature, the absorbance was measured at 595 nm and protein quantification was performed.

[0036]The SDS polyacrylamide gel electrophoresis of the lysate which contains equivalent weight of protein by the buffer system (Laemmli, N. K., "Nature", 283:pp. 249-256, 1970) of Laemmli was performed using the sample which performed protein quantification. Blocking following blotting and it was performed after electrophoresis. Namely, a protein transfer device (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad, catalog number 170-3946) is used, At the room temperature, gel was stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), and blotting was performed 100V for 3 hours. As a blotting buffer, The trisglycine buffer which consisted of 0.025M tris and 0.192M glycine, and was adjusted the pH to 8.5 (catalog number Tris Gly Running and Blotting Buffer;Enprotech, the U.S. Massachusetts state) The buffer which prepared methyl alcohol in addition to SA100034 so that it might become 20% was used. After blotting, the nitrocellulose membrane was incubated for 30 minutes at the room temperature in the 10% skim milk (Snow Brand Milk Products)-PBS (-) solution, and nonspecific combination was blocked.

[0037]The anti human HSP60 mouse monoclonal antibody (StressGen, Victoria, B.C., Canada, catalog number SPA-806) performed primary antibody reactions on the nitrocellulose membrane after blocking. This anti human HSP60 mouse monoclonal antibody, It is the antibody

which produced as immunogen Homo sapiens HSP60 produced by the recombinant DNA method using Escherichia coli ("J. Exp. Med." 175, 1805-1810, 1992),It reacts to mammals HSP60 (primates HSP60, mouse HSP60, rat HSP60, and hamster HSP60) specifically ("J. Exp. Med." 175, 1805-1810, 1992). Localization of the epitope which this anti human HSP60 mouse monoclonal antibody recognizes is carried out into the amino acid sequence which consists of the 383rd - the 447th amino acid residue of Homo sapiens HSP60 amino acid sequence ("J. Exp. Med." 175, 1805-1810-1992). After primary antibody reactions, exchange every [a for / 5 minutes], and a solution by PBS (-), and a slow locking shaker performs two washing, Every [a for / 15 minutes] and a solution were exchanged with Tween20 (Bio-Rad, catalog number 170-6531) solution PBS (-)-0.1%, and four washing was performed. Eventually, every [a for / 5 minutes] two washing was performed by PBS (-).

[0038]Secondary antibody reactions were performed after the end of washing for 2 hours using 5 ml of antibody solutions which diluted and prepared the peroxidase-labeling goat anti-mouse IgG antibody (CAPPEL, catalog number 55550) 5000 times with the PBS (-) solution which contains skim milk 2%. After ending reaction, about the nitrocellulose membrane, the solution was changed for 5 minutes at a time with the PBS (-) solution, the solution was changed for 15 minutes at a time with Tween20 solution 2 times and also PBS(-)-0.1%, and the slow locking shaker performed five washing. Finally the PBS (-) solution performed every [a for / 5 minutes] two washing. After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Western blotting detectionreagent; Amersham and catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After incubating for 1 minute, the excessive detecting reagent was removed, the nitrocellulose membrane was wrapped in the lap, the reaction surface was stuck to the X-ray film (Kodak X-OMAT, AR, catalog number 1651454), was exposed, was developed, and the existence of HSP60 was examined. A result is shown in Table 1. "**" means among front that HSP60 expression amount decreased compared with contrast.

[0039]

[Table 1]

Cancer type Cancer cell Quercetin Rutin Catechin BAIKA lane uterus HeLa S3 ** kidney ACHN ** prostate gland DU 145 **** prostate gland PC-3 ** milk MCF7 ** ** **[0040] The band of molecular weight abbreviation 60kD was detected one by the control test, i.e., the cell which did not add flavonoid. It combined with said anti human HSP60 mouse monoclonal antibody, and the molecular weight marker (egg white ovalbumin and bovine serum albumin) determined the molecular weight. Catechin controlled the manifestation of HSP60 in kidney cancer cell strain ACHN and breast cancer cell line MCF7 as shown in Table 1. Quercetin controlled the manifestation of HSP60 in uterus cancer cell line HeLaS3, prostate cancer cell line DU 145, prostate cancer cell line PC-3, and breast cancer cell line MCF7. Rutin controlled the manifestation of HSP60 in breast cancer cell line MCF7. The BAIKA lane controlled the manifestation of HSP60 in prostate cancer cell line DU145. That is, catechin, quercetin, rutin, and a BAIKA lane can be concluded to have the activity of the synthetic inhibitor which controls the manifestation of HSP60.
[0041]
[Effect of the Invention]As explained in full detail above, flavonoid has the activity of the synthetic inhibitor which controls the manifestation of protein belonging to HSP60 intracellular family. Therefore, by prescribing flavonoid for the patient, protein belonging to HSP60 family can make the physiological status of the patient of autoimmune diseases (for example, type I diabetes mellitus, rheumatoid arthritis, etc.) who participates in the onset able to improve effectively, and can treat said illness effectively, for example.

[Translation done.]